Please replace the first paragraph on page 2 with the following:

--Novel ways of monitoring specific modulation of intracellular pathways in intact, living cells are assumed to provide new opportunities in drug discovery, functional genomics, toxicology, patient monitoring, etc.—

Please replace the first full paragraph on page three, starting at line 5, with the following:

--In an alternative method, protein kinase A activity has been measured in living cells by chemical labelling one of the kinase's subunits (Adams *et al.* 1991). The basis of this methodology is that the regulatory and catalytic subunit of the purified protein kinase A is labelled with fluorescein and rhodamine, respectively. At low cAMP levels, protein kinase A is assembled in a heterotetrameric form which enables fluorescence resonance energy transfer between the two fluorescent dyes. Activation of protein kinase A leads to dissociation of the complex, thereby eliminating the energy transfer. A disadvantage of this technology is that the labelled protein kinase A has to be microinjected into the cells of interest. This highly invasive technique is cumbersome and not applicable to large scale screening of biologically active substances. A further disadvantage of this technique, as compared to the presented invention, is that the labelled protein kinase A cannot be inserted into organisms/animals as a transgene.—

Please replace the third paragraph on page 11, starting at line 10, with the following:

--In one embodiment, the biologically active polypeptide encoded by the nucleic acid construct is a mitogen-activated serine/threonine protein kinase or a part thereof capable of changing cellular

localisation upon activation. In preferred embodiments, the biologically active polypeptide encoded by the nucleic acid constructs is an ERK1-F64L-S65T-GFP fusion or an EGFP-ERK1 fusion.—

Please replace the first paragraph on page 12 with the following:

--The present invention relates to a method that may be used to establish a screening program for the identification of biologically active substances that directly or indirectly affect intracellular signalling pathways and because of this property are potentially useful as medicaments. Based on measurements in living cells of the redistribution of spatially resolved luminescence from luminophores which undergo a change in distribution upon activation or deactivation of an intracellular signalling pathway, the result of the individual measurement of each substance being screened indicates its potential biological activity.—

Please replace the last paragraph on page 13, lines 28-31, which continues onto page 14, lines 1-4, with the following:

---Design of gene-specific primers. Inspection of the sequence of the gene allows design of gene-specific primers to be used in a PCR reaction. Typically the top-strand primer encompasses the ATG start codon of the gene and the following approx. 20 nucleotides, while the bottom-strand primer encompasses the stop codon and the approx. 20 preceding nucleotides, if the gene is to be fused behind GFP, i.e. a GFP -"GeneX" fusion. If the gene is to be fused in front of GFP, i.e. a "GeneX-GFP" fusion, a stop codon must be avoided. Optionally, the full length sequence of GeneX may not be used in the fusion, but merely the part which localizes and redistributes the GeneX in response to a signal.—

Please replace the third paragraph on page 18, lines 29-31, which continues onto page 19, lines 1-2, with the following:

--The number of ways of extracting a single value from an image is extremely large, and thus an intelligent approach must be taken to the initial step of reducing this number to a small, finite number of possible procedures. This is not to say that the procedure arrived at is necessarily the best procedure - but a global search for the best procedure is simply out of the question due to the sheer number of possibilities involved.—

Please replace the first paragraph on page 22 with the following:

--The terms "image processing" and "image analysis" are used to describe a large family of digital data analysis techniques or combination of such techniques which reduce ordered arrays of numbers (images) to quantitative information describing those ordered arrays of numbers. When said ordered arrays of numbers represent measured values from a physical process, the quantitative information derived is therefore a measure of the physical process.—

Please replace the third paragraph on page 22, lines 15-28, with the following:

--The term "mammalian cell" is intended to indicate any living cell of mammalian origin. The cell may be an established cell line, many of which are available from The American Type Culture Collection (ATCC, Virginia, USA) or a primary cell line with a limited life span derived from a mammalian tissue, including tissues derived from a transgenic animal, or a newly established immortal cell line derived from a mammalian tissue including transgenic tissues, or a hybrid cell or

cell line derived by fusing different cell types of mammalian origin, e.g. hybridoma cell lines. The cells may optionally express one or more non-native gene products, e.g. receptors, enzymes, enzyme substrates, prior to or in addition to the fluorescent probe. Preferred cell lines include, but are not limited to, those of fibroblast origin, e.g. BHK, CHO, BALB, or of endothelial origin, e.g. HUVEC, BAE (bovine artery endothelial), CPAE (cow pulmonary artery endothelial) or of pancreatic origin, e.g. RIN, INS-1, MIN6, bTC3, aTC6, bTC6, HIT, or of hematopoietic origin, e.g. AtT20, PC12, GH3, muscle origin, e.g. SKMC, A10, C2C12, renal origin, e.g. HEK 293, LLC-PK1.—

Please delete paragraph 3 on page 24, line 17, which begins "The phrase "back-tracking" and ends with "to indicate".

Please replace the third paragraph on page 27, lines 15-25, with the following:

--Figure 6. Parallel dose response analyses of forskolin induced cAMP elevation and PKAc-F64L-S65T-GFP redistribution. The effects of buffer or 5 increasing concentrations of forskolin on the localisation of the PKAc-F64L-S65T-GFP fusion protein in CHO/ PKAc-F64L-S65T-GFP cells, grown in a 96 well plate, were analysed as described above. Computing the ratio of the SD's of fluorescence micrographs taken of the same field of cells, prior to and 30 min after the addition of the forskolin, gave a reproducible measure of PKAc-F64L-S65T-GFP redistribution. The graph shows the individual 48 measurements and a trace of their mean ± s.e.m at each forskolin concentration. For comparison, the effects of buffer or 8 increasing concentrations of forskolin on

[cAMP], were analysed by a scintillation proximity assay of cells grown under the same conditions. The graph shows a trace of the mean ± s.e.m of 4 experiments expressed in arbitrary units.—

Please replace the first paragraph under the heading "Monitoring activity of PKA activity in real time:" on page 31 continuing onto page 32, lines 1-2, with the following:

--Image acquisition of live cells was gathered using a Zeiss Axiovert 135M fluorescence microscope fitted with a Fluar 40X, NA: 1.3 oil immersion objective and coupled to a Photometrics CH250 charged coupled device (CCD) camera. The cells were illuminated with a 100 W HBO arc lamp. In the light path was a 470±20 nm excitation filter, a 510 nm dichroic mirror and a 515±15nm emission filter for minimal image background. The cells were kept and monitored to be at 37°C with a custom built stage heater.—

Please replace paragraph b) under Example 18, lines 20-25, with the following:

--b) The human Stat5 gene (GenBank Accession Number: L41142) was amplified using PCR according to standard protocols with primers Stat5-top (SEQ ID NO:30) and Stat5-bottom/-stop (SEW ID NO:31). The PCR product was digested with restriction enzymes Bgl2 and Acc651, and ligated into pEGFP-N1 (Clontech, Palo Alto; GenBank Accession Number U55762) digested with Bgl2 and Acc651. This produced a Stat5-EGFP fusion (SEQ ID NO:78 and 79) under the control of a CMV promoter.--